HLA and Multiple Myeloma among Black and White Men: Evidence of a Genetic Association

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Abstract

To evaluate the possibility that genetic factors contribute to the excess rates of multiple myeloma among blacks, serological typing of human leukocyte antigens (HLA) was conducted for black and white male patients and controls who participated in a large population-based case-control interview study. Forty-six black cases, 88 black controls, 85 white cases, and 122 white controls were typed for the Class I antigens (HLA-A, -B, -C) and for the Class II antigens (HLA-DR, HLA-DQ). Black cases had significantly higher gene frequencies than black controls for Bw65, Cw2, and DRw14, while white cases had higher gene frequencies than white controls for A3 and Cw2 and blanks at the DR and DQ loci. Further analysis of the association between Cw2 and multiple myeloma revealed relative risks of 5.7 (95% confidence interval = 1.5–26.6) and 2.6 (95% confidence interval = 1.0–7.2) for blacks and whites, respectively. The frequency of Cw2 in black and white controls was similar. These findings suggest that the Cw2 allele enhances the risk of myeloma in blacks and whites but do not explain the higher incidence of this cancer among blacks. The study also suggests that undefined DQ antigens may play an etiological role, supporting the need for further research into the immunogenetic determinants of myeloma.

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Introduction

Multiple myeloma is the only hematopoietic malignancy that occurs more frequently among U.S. blacks than whites (1). Little is known about the etiology of myeloma, but it has been postulated that genetic factors may contribute to the racial differential. In the early 1970s, several hospital-based studies of myeloma attempted to establish genetic susceptibility by evaluating HLA[4] (2–7). At that time, the Class II gene loci had not yet been discovered, and only two of the three Class I gene loci (HLA-A, -B), with a limited number of antigens, had been identified. In addition, small sample sizes, a lack of black cases, inadequate control groups, and inappropriate statistical testing led to difficulties in the interpretation of some of the findings. In 1983, the first report of an excess of a specific HLA type (Cw5) among black myeloma patients was published (8). To pursue this lead and overcome the limitations of previous studies, HLA serological typing for the Class I antigens (HLA-A, -B, -C) and for the Class II antigens (HLA-DR, -DQ) was included in a population-based case-control interview study of myeloma among U.S. blacks and whites.

Methods

Study Population. The original study population consisted of all histologically confirmed black and white patients with multiple myeloma, aged 30–79, newly diagnosed from August 1, 1986 to April 30, 1989, who resided in one of three areas of the United States covered by population-based cancer registries. These included two counties in Georgia, three counties in Michigan, and 10 counties in New Jersey. Subjects were identified from pathology, hematology, outpatient, and tumor registry records. Cases of pancreatic, esophageal, and prostatic cancer also were identified in the study.

For logistical and financial reasons, one control group was selected to be used in the risk analysis of all four cancer types. Random digit dialing techniques (9) were used in selecting controls aged 30–64, and computerized files of Medicare recipients from the Health Care Financing Administration were used to select controls aged 65–79. The number of population controls to be sampled was based on the age-sex-race distribution of previously diagnosed cases from each study area.

Interviews. In-person interviews were conducted by trained interviewers, usually in the subjects' homes. A modular questionnaire was developed so that subjects

[4] The abbreviations used are: HLA, human leukocyte antigen(s); RR, relative risk; CI, confidence interval.

with a particular cancer type were asked questions specific for that cancer, whereas control subjects were asked all questions. The sections for myeloma patients elicited information on sociodemographic characteristics, medical history, occupational history, usual adult diet, alcohol consumption, smoking habits, and other potential risk factors.

Blood Collection. Blood collection was limited to male myeloma patients and a sample of male controls due to logistical and financial constraints. Prior to conducting an interview, permission was obtained from the patient's physician, both to contact the subject for an interview and to collect blood. Once the interview was completed, the subject was asked to participate in the blood collection. A phlebotomist then followed up with a phone call to answer any questions and arrange for blood to be drawn at the subject's home.

Prior to drawing blood, the phlebotomist obtained the subject's signed consent and administered a brief questionnaire about medical conditions (e.g., hemophilia) and medication use (e.g., Coumadin), which were considered contraindications for drawing blood. A heparinized syringe was used to draw approximately 40 ml of blood from participating subjects. The blood was then injected into a transfer pack that contained RPMI 1640 culture media and shipped by express mail to the typing lab at Howard University Hospital (Washington, D.C.) so that analysis could be performed within 36 h after the blood was drawn.

The presence of HLA Class I (*i.e.*, -A, -B, -C) and Class II (*i.e.*, -DR, -DQ) antigens was determined in a two-stage complement-dependent microcytotoxicity assay (10). Mononuclear cells separated by Ficoll-Hypaque density gradient centrifugation from heparinized peripheral blood were used to determine Class I antigens. B-cells separated from mononuclear cells by the nylon wool technique were used to determine Class II antigens (11). HLA designations were based on the nomenclature formulated at the Ninth International Histocompatibility Workshop and Conference in Munich and Vienna, 1984. The set of antisera used was obtained from various commercial suppliers and included the following: HLA-A1, 2, 3, 9, 23, 24, 10, 25, 26, w34, w66, 11, w19, 29, 30, 31, 32, w33, 28, w68, w69, w36; HLA-B5, 51, w52, 7, 8, 12, 44, 45, 13, 14, w64, w65, 15, w62, w63, 16, 38, 39, 17, w57, w58, 18, 21, 49, w50, w22, w54, w55, w56, 27, 35, 37, 40, w60, w41, w42, w46, 47, w48, w53, w59, w67, w70, w71, w73, w4, w6; HLA-Cw1, w2, w3, w4, w5, w6, w7, w8; HLA-DR1, 2, 3, 4, 5, 11, 12, w6, w13, w14, 7, w8, w9, w10, w52, w53; HLA-DQw1, w2, w3.

Statistical Methods. Statistical analyses of the HLA data assumed the Hardy-Weinberg equilibrium and used maximum likelihood methods to estimate the gene frequencies from the phenotypic data (12, 13). In several instances, the estimated gene frequency for blanks (or as yet undefined alleles) is greater than zero, even when the observed number of double blanks is zero (14). Omnibus χ^2 tests were calculated to compare the profile of gene frequencies between black and white controls and between cases and controls by race (12, 13).

In addition, individual χ^2 tests were calculated for each of the specific alleles (including blanks) at each of the five loci that resulted from the HLA typing of the cases or the controls. For the case-control comparisons, these tests were combined over race by using the signed summation of χ technique (15). All *P*-values reported are two-tailed. Individual test *P*-values were adjusted for multiple comparisons in two ways: the intralocus Bonferroni correction, in which individual *P*-values are multiplied by the number of alleles at the particular locus; and the overall Bonferroni correction, in which the intralocus Bonferroni *P*-value is multiplied by five, the number of loci being typed (16).

For those gene frequencies judged significant by the above criteria, additional analyses of the RR, as approximated by the odds ratio, were performed. In these instances, exact conditional estimates, statistical tests, and confidence intervals were used (17). In conjunction with these analyses, estimates of the attributable risk and their approximate confidence limits were calculated (18).

Results

Description of Study Subjects. Among the 474 male cases and 562 male controls preselected for participation in the blood component, interviews were completed for 295 cases and 426 controls. The interview response rates were highest for black control subjects (70.9%) followed by white control subjects (69.0%), white cases (63.8%), and black cases (59.0%). The lower response rates among the cases were due primarily to the high percentage of blacks (28.8%) and whites (20.8%) who died prior to the interview. Refusal rates among cases were fairly low: 2.6% for blacks and 5.7% for whites.

Table 1 presents the blood collection participation results for the interviewed male cases and controls by race. Among the black subjects, 48 cases (52.2%) and 99 controls (61.5%) agreed to have blood drawn. Among the white subjects, 92 cases (45.3%) and 143 controls (54.0%) agreed to participate in the blood collection.

Description of the HLA Data. Among the control subjects who had blood drawn, one black subject and one white subject had their blood samples lost or destroyed during shipping. In addition, the blood samples from 12 blacks (2 cases, 10 controls) and 27 whites (7 cases, 20 controls) did not yield suitable typing results primarily due to cell damage during shipping. For the remaining cases (46 blacks, 85 whites) and control subjects (88 blacks, 122 whites), over 97% had successful typing of Class I antigens, while over 80% of the cases and 92% of the controls were successfully typed for Class II antigens.

Comparison of the Controls by Race. The omnibus χ^2 tests for each of the five loci and individual χ^2 tests for

Table 1 Blood collection participation results among interviewed cases and controls by race

Result	Blacks				Whites			
	Cases $n = 92$		Controls $n = 161$		Cases $n = 203$		Controls $n = 265$	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Blood drawn	48	(52.2)	99	(61.5)	92	(45.3)	143	(54.0)
Deceased	1	(1.1)	0	(0.0)	4	(2.0)	1	(0.4)
Too ill	21	(22.8)	11	(6.8)	62	(30.5)	32	(12.1)
Refused	20	(21.7)	41	(25.5)	39	(19.2)	80	(30.2)
Unavailable	2	(2.2)	10	(6.2)	6	(3.0)	9	(3.4)

Table 2 Comparison of estimated individual gene frequencies with significant ($P < 0.01$) differences or blank alleles between black and white controls

	Blacks			Whites			P	Omnibus test	
	G[a]	n	$p' \pm$ SE	G	n	$p' \pm$ SE	Individual	χ^2	P
A locus (22 alleles)		$N = 85$			$N = 118$			64.95	2×10^{-6}
A2	19	3	12.0 ± 2.5	54	6	25.4 ± 2.8	0.001[b]		
A11	2	0	1.2 ± 0.8	17	0	7.2 ± 1.7	0.004		
A24	3	1	1.8 ± 1.0	19	0	8.1 ± 1.8	0.006		
Aw34	12	1	7.2 ± 2.0	4	0	1.7 ± 0.8	0.006		
Blank		0	7.6 ± 2.5		0	0.0 ± 1.1	0.002[b]		
B locus (39 alleles)								70.36	0.001
B17	12	1	7.2 ± 2.0	3	0	1.3 ± 0.7	0.002		
Blank		1	10.2 ± 2.6		0	5.8 ± 1.9	0.160		
C locus (9 alleles)								12.98	0.110
Cw2[c]	4	2	2.4 ± 1.2	9	3	3.9 ± 1.3	0.410		
Blank		24	51.6 ± 4.0		25	40.0 ± 3.4	0.030		
DR locus (15 alleles)		$N = 81$			$N = 112$			23.02	0.060
DR4	7	2	4.5 ± 1.7	29	3	13.8 ± 2.3	0.003[b]		
Blank		0	10.2 ± 3.0		0	3.6 ± 2.0	0.052		
DQ-locus (4 alleles)								14.40	0.002
DQw3	22	3	14.0 ± 2.8	55	13	27.9 ± 3.2	0.002[b]		
Blank		7	21.7 ± 4.2		4	10.4 ± 3.2	0.030		

[a] G, antigen frequency; n, number of individuals reacting to a single antigen only; p', estimated gene frequency in percent using maximum likelihood estimates from phenotypic data.

[b] Intralocus Bonferroni adjustment for multiple comparisons significant at $P < 0.05$.

[c] Cw2 is included here because of its association with multiple myeloma (see Table 5).

each allele were conducted to compare the gene frequencies between black and white control subjects (Table 2). The omnibus χ^2 tests revealed significant differences between black and white controls at the A, B, and DQ loci. Analyses of the individual alleles of the A locus revealed a substantial and significant excess of A2 among white controls compared to black controls even after intralocus Bonferroni adjustment ($P = 0.02$). This finding was somewhat compensated for by an elevated frequency of Aw34 (intralocus Bonferroni; $P = 0.13$) and blanks at the A locus (intralocus Bonferroni; $P = 0.04$) among black controls. At the DR and DQ loci, white controls had a significantly higher frequency of DR4 (intralocus Bonferroni; $P = 0.04$) and DQw3 (intralocus Bonferroni; $P = 0.008$) than black controls, while blank frequencies were somewhat higher among the blacks. The frequency of Cw2 among black and white controls was not significantly different.

Comparison of Cases and Controls by Race. The omnibus χ^2 test, used to compare case-control gene frequencies for each of the five loci, revealed no significant differences between black cases and control subjects. For whites, a significant case-control difference at the DQ locus was evident ($P = 0.01$).

Table 3 presents specific alleles at which case or control differences were significant at the 0.05 level (or nearly significant) for one or both races. Among the black subjects, cases had a higher frequency of Bw65, Cw2, and DRw14 alleles, whereas controls had a higher frequency of blanks at the A locus. It is noteworthy that none of the black control subjects had Bw65 or DRw14 antigens. Among the white subjects, cases had a higher frequency than controls for A3, Cw2, and blanks at the DR and DQ loci, whereas the controls had a higher frequency of Bw53. Case-control differences in the frequency of the Cw2 allele yielded a statistically significant intralocus Bonferroni P-value of 0.03 for blacks, and a P-value of 0.27 for whites.

Adjusting for race using the summation χ test showed that cases had a significantly higher frequency of Cw2 antigen than controls ($P = 0.0002$). This difference remained significant after adjustment for multiple comparisons using both the intralocus Bonferroni correction ($P = 0.002$) and the overall Bonferroni correction ($P = 0.01$). This higher frequency of Cw2 among cases was somewhat compensated for by a lower frequency of blanks at this locus. At the DR and DQ loci, significantly higher frequencies of blanks were found among cases than controls, after adjusting for race. When these differences were adjusted using the intralocus Bonferroni correction, the P-values were no longer significant for DR blanks ($P = 0.57$) or DQ blanks ($P = 0.07$).

The preceding analysis assumed that the Hardy-Weinberg law holds for both the cases and controls in the two races. In fact, among the white cases at the C locus, the usual χ^2 goodness of fit test rejected this assumption ($P = 0.003$). We therefore confirmed our results for Cw2 by analyzing the proportions with and without Cw2 by traditional relative risk methods (Table 4). Blacks with Cw2 had an estimated RR of 5.7 (95% CI = 1.5–26.6), and for whites the RR was 2.6 (95% CI = 1.0–7.2). These RRs were not significantly different.

The RR for both races combined was highly significant ($P = 0.0006$) even after adjustment for multiple comparisons (intralocus $P = 0.005$; overall Bonferroni corrected $P = 0.027$). The multiple myeloma risk attributed to Cw2 was estimated to be 18.4% (95% CI = 8.5–35.4) among blacks and 11.1% (95% CI = 4.3–25.6) among whites. The mean age at diagnosis was similar for cases with and without Cw2; thus the presence of Cw2

Table 3 Comparison of estimated individual gene frequencies with significant (or nearly significant) differences ($P < 0.05$) between cases and controls by race

	Blacks								Whites								$\Sigma\chi$	(P)
	Cases			Controls			% diff	p	Cases			Controls			% diff	p		
	G[a]	n	p′ ± SE	G	n	p′ ± SE			G	n	p′ ± SE	G	n	p′ ± SE				
A locus (22 alleles)																		
A3	9	1	11.1 ± 3.3	18	2	11.1 ± 2.5	+0.0	1.00	33	5	22.6 ± 3.2	31	1	13.6 ± 2.2	+9.0	0.02	+1.61	(0.095)
Blank		0	0.0 ± 1.8		0	7.6 ± 2.5	−7.6	0.05		0	0.3 ± 1.4		0	0.0 ± 1.0	+0.3	0.84	−1.27	(0.204)
B locus (43 alleles)																		
Bw53	5	0	5.6 ± 2.4	8	2	4.9 ± 1.7	+0.7	0.81	1	0	0.6 ± 0.6	8	2	3.6 ± 1.2	−3.0	0.05	−1.24	(0.214)
Bw65	3	0	3.3 ± 1.9	0	0	0.0 ± 0.0	+3.3	0.02	1	0	0.6 ± 0.6	3	1	1.3 ± 0.7	−0.7	0.48	+1.17	(0.242)
C locus (9 alleles)																		
Cw2	10	4	11.7 ± 3.5	4	2	2.4 ± 1.2	+9.3	0.002	15	3	9.1 ± 2.3	9	3	3.9 ± 1.3	+5.2	0.03	+3.71	(0.0002)[b]
Blank		8	38.1 ± 5.5		24	51.6 ± 4.0	−13.5	0.05		15	35.7 ± 3.9		25	40.0 ± 3.4	−4.3	0.41	−1.97	(0.048)
DR locus (15 alleles)																		
DRw14	3	1	4.3 ± 2.4	0	0	0.0 ± 0.0	+4.3	0.007	1	1	0.7 ± 0.7	3	0	1.3 ± 0.8	−0.6	0.58	+1.51	(0.132)
Blank		0	11.4 ± 4.4		0	10.2 ± 3.0	+1.2	0.82		0	13.6 ± 3.4		0	3.6 ± 2.0	+10.0	0.007	+2.07	(0.038)[c]
DQ locus (4 alleles)																		
DQw3	13	4	18.9 ± 4.8	22	3	14.0 ± 2.8	+4.9	0.36	24	7	18.5 ± 3.4	55	13	27.9 ± 3.2	−9.4	0.06	−0.70	(0.484)
Blank		3	28.3 ± 6.3		7	21.7 ± 4.2	+6.6	0.38		5	24.0 ± 4.6		4	10.4 ± 3.2	+13.6	0.013	+2.37	(0.018)[d]

[a] G, antigen frequency; n, number of individuals reacting to a single antigen only; p′, estimated gene frequency using maximum likelihood estimates from phenotypic data; % diff, difference in gene frequency between cases and controls.
[b] Intralocus Bonferroni adjusted $P = 0.002$; overall Bonferroni-adjusted $P = 0.01$.
[c] Intralocus Bonferroni adjusted $P = 0.57$.
[d] Intralocus Bonferroni adjusted $P = 0.07$.

does not appear to be associated with early onset of myeloma.

The nearly significant excess of the blank gene frequency at the DQ locus among cases (both races combined) raised the possibility that an as yet unspecified allele may have accounted for the increased risk of myeloma due to Cw2. We attempted to determine whether the presence of such a gene, represented by a blank or only one antigen at the DQ locus, was interacting with Cw2 to increase disease susceptibility. Following Simons *et al.* (19), we cross-classified the cases and controls by Cw2 and the presence of 0, 1, or 2 DQ antigens and then evaluated as a joint risk factor both having Cw2 and less than 2 antigens at the DQ locus. The overall RR for this combination factor was 4.6, somewhat higher than the RR for Cw2 alone (RR = 3.5). A RR of 4.6 was also seen for whites and blacks, which was an increase over the risk for Cw2 alone among whites and a decrease in the risk among blacks.

Discussion

This was the first population-based study to conduct HLA serological typing on black and white myeloma cases and controls as a means of evaluating genetic susceptibility to this cancer. The most intriguing finding was the significant association of multiple myeloma with the HLA-Cw2 antigen among both black and white males. Attributable risk calculations suggested that over 18% of the black male myeloma cases and 11% of the white male myeloma cases may be due to the presence of the Cw2 antigen.

HLA antigens at the C locus have previously been evaluated in only one study in which 22 black myeloma patients from two Louisiana clinics were compared to 138 laboratory controls (8). Both studies found a link between an HLA-C-antigen and multiple myeloma in black men, although the specific C-antigen differed between the studies (Cw2 in the present study and Cw5 in the earlier one). The reason(s) for this specific C-antigen difference is not known but may reflect heterogeneity in the disease and/or population or differences in study design.

Since multiple myeloma is a cancer of the plasma cells (which produce antibodies), HLA-associated genes may be involved etiologically through the control of immune responses. There are several possible indirect

Table 4 RR and attributable risk of multiple myeloma associated with HLA-Cw2 by race

	Cases (n/N)[a]	Controls (n/N)	RR	(95% CI)	AR	(95% CI)
Blacks	10/45	4/85	5.7	(1.5–26.6)	18.4%	(8.5–35.4)
Whites	15/84	9/118	2.6	(1.0–7.2)	11.1%	(4.3–25.6)
Combined analysis[b]			3.5	(1.6–7.7)[c]	13.6%	(7.4–23.8)

[a] n, subjects with Cw2; N, total number of subjects; AR, attributable risk.
[b] Test for difference in RR between races: $P = 0.46$.
[c] Intralocus Bonferroni adjusted $P = 0.0054$; overall Bonferroni adjusted $P = 0.027$.

mechanisms by which HLA alleles may be associated with disease susceptibility. If viruses are etiologically important, the HLA antigen may serve as a cell-surface receptor for a transforming virus. From an immune surveillance perspective, if sequence homology were detected between Cw2 and a peptide in a putative transforming virus, such structural similarity could compromise immune recognition of the virus, favor survival of transformed cells, and explain the association of the C allele with myeloma. At least two Cw2 alleles (Cw2.1 and Cw2.2) have been identified by DNA sequencing (20, 21). Additional studies are needed to determine whether the risk of myeloma is specifically associated with a particular Cw2 allele.

Another possibility is that our observed association is due to a gene closely linked or in linkage disequilibrium with Cw2. Since the risk of myeloma was further increased (primarily for whites) when Cw2 was associated with an undefined DQ antigen, a likely candidate would be a gene within or closely linked to the DQ subregion. Some DQ blanks have been defined as DQw4 (formerly "WA") for a large proportion of blacks and whites (22), and DNA sequence data have shown at least 8 DQA1 alleles and 13 DQB1 alleles at the DQ locus. Finer definition of the DQ allele is needed to determine the DQ antigen possibly associated with Cw2.

A number of methodological issues need to be considered when evaluating HLA associations with disease. The observed Cw2 myeloma association was not an a *priori* hypothesis and may be due to chance. However, the consistency of the finding for both races, even after extreme adjustment for multiple comparisons, strongly suggests that it is not spurious. Although our study was population based, with rapid ascertainment of all incident cases of myeloma from three geographic areas, short survival greatly reduced participation rates in the blood-drawing component. Thus, the poor prognosis of this malignancy resulted in the elimination of cases surviving less than 4 months. Although it is possible that the Cw2 antigen may be a predictor of survival, this does not seem to be the explanation for our Cw2 finding. For example, when we recalculated the myeloma risk associated with Cw2 by including the deceased black cases in the analysis and assuming that they all lacked the Cw2 antigen, the risk remained significantly elevated (RR = 3.8; 95% CI = 1.0–17.4).

Our control subjects were selected to be representative of the general population residing in the three study areas. Interview response rates were generally high; however, more than 25% of these controls refused to have blood drawn. Nevertheless, the gene frequency data for our black and white subjects who gave blood were fairly consistent with reported population frequencies based on International Workshop data (23), suggesting that the low blood participation rates among controls did not introduce a detectable bias.

The possibility also exists that other factors (potential confounders) associated with both myeloma and Cw2 may have distorted the magnitude of our HLA finding. To date, the amount of information on risk factors for myeloma is limited. It is, therefore, premature to speculate on how other factors may have influenced our results.

In conclusion, an excess frequency of HLA-Cw2 among cases may contribute to the increased incidence of multiple myeloma among blacks. Because Cw2 is present in about the same frequency in black and white control subjects, this allele cannot fully account for this excess. Environmental factors as yet unknown may increase the risk of myeloma among those individuals predisposed to developing this cancer. Our findings also raise the possibility that unidentified alleles, particularly at the DQ locus, may play an etiological role. Thus, there is a need for further research into environmental and immunogenetic determinants of multiple myeloma.

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